Regulation of hepatic carnitine palmitoyltransferase activity during the foetal–neonatal transition

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Overt carnitine palmitoyl transferase (CPT_1) activity was measured in liver mitochondria from foetal rats (21 days gestation) and from neonatal rats (1 day post-partum). Birth was accompanied by a 6-fold increase in CPT_1 activity, a 14-fold decrease in sensitivity to inhibition by malonyl CoA and an increase in the n_H and the $S_{0.5}$ for palmitoyl CoA. The activity of latent enzyme (CPT_2) was unaffected at birth.

Carnitine palmitoyltransferase *Malonyl CoA* *Foetal–neonatal transition* *Thyroid status*

1. INTRODUCTION

Many mammals, including the human and the rat, are subjected to a major change in nutrition at birth. In the foetal animal glucose, provided from the mother via the placenta, is the major oxidizable substrate. Non-esterified fatty acids are not considered to be a fuel for the foetus since they do not readily cross the placental barrier and their concentration is low in foetal blood [1,2]. Furthermore, in normal circumstances, maternally-derived ketone bodies only provide a small proportion of foetal energy requirements [3]. At birth the transplacental diet, high in carbohydrate, is replaced by the high-fat milk diet of suckling. Marked hyperketonaemia is observed in new born infants [4,5] and in other mammals, including the rat [6–9]. During the suckling period ketone bodies become major fuels for certain extrahepatic tissues including the brain [8]. It is therefore necessary for the neonatal liver to establish the capability for fatty acid oxidation and ketogenesis. A number of changes occur near to the time of birth which may contribute to the development of this capacity:

(i) Increased levels of plasma non-esterified fatty acids [10];
(ii) An increase in the hepatic carnitine content [10,11];
(iii) Increased activity in hepatic mitochondria of carnitine palmitoyltransferase [7,12–16], β-oxidation enzymes [15] and enzymes of the hydroxymethylglutaryl CoA pathway [17];
(iv) A substantial decrease in the plasma insulin/glucagon ratio [6,18];
(v) Increased gluconeogenesis [19,20] which may reduce oxaloacetate availability and hence divert acetyl CoA metabolism towards ketogenesis [21].

The overt activity of carnitine palmitoyltransferase (CPT_1) in mitochondria from rat liver is potently inhibited by malonyl CoA [22,23]. Since the hepatic malonyl CoA content and the rate of fatty acid synthesis are closely correlated [23,24], this effect is thought to contribute to the mechanism whereby rates of hepatic fatty acid synthesis and oxidation are inversely related in a number of states [23]. It has also become apparent that the sensitivity of liver CPT_1 to malonyl CoA is altered in some conditions [25–30]. In essence, the sensitivity of CPT_1 to malonyl CoA inhibition is higher and lower respectively in those states with increased and decreased hepatic malonyl CoA contents [29] thereby expanding the range of response of the enzyme to alterations in [malonyl CoA]. Although we are unaware of any actual measurements, it is likely that the hepatic malonyl CoA

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content fails during the foetal–suckling neonatal transition since at this time there is a substantial decrease in fatty acid synthesis both in vitro [31,32] and in vivo [7,33].

Here, we investigated changes in the activity and properties of hepatic CPT_1 during the foetal–neonatal transition, paying particular regard to the sensitivity of the enzyme to malonyl CoA.

2. MATERIALS AND METHODS

Sources of chemicals are described in [25,30]. Liver mitochondria were isolated as in [34] using the following as sources:

(i) Single livers from fed pregnant Sprague-Dawley rats (20–21 days gestation). These had been mated as virgins when aged 13–15 weeks.
(ii) 10–12 pooled foetal livers (male and female) taken from the pups of the mothers used in (i).
(iii) 6–8 pooled livers from female rat pups on the first day after birth. These were allowed to suckle until the time of sacrifice.

Mitochondria were finally suspended in 0.3 M sucrose containing 10 mM Tris–HCl buffer (pH 7.4) and 1 mM EGTA and stored in ice. Mitochondrial protein was determined by the method in [35].

CPT_1 assays were performed within 30 min of the isolation of mitochondria. Aliquots (50 μl) of intact mitochondria were incubated at 25°C for 4 min in 1 ml assay mixtures to follow the incorporation of [^{3}H]carnitine into palmitoyl-[^{3}H]carnitine as in [30,36]. Mitochondrial protein concentrations in these assays were: foetal, 243 ± 26; neonatal, 167 ± 18; pregnant maternal, 210 ± 17 (means ± SEM as μg/ml for 4 separate expt in each case).

Total CPT activity was measured similarly using 25 μl aliquots of mitochondria that had been sonicated for 2 min at 2°C.

CPT_2 activity was calculated by subtraction of CPT_1 from total CPT.

Assays were linear with time and mitochondrial protein concentration.

3. RESULTS AND DISCUSSION

Fig. 1 shows that CPT_2 maximal activity relative to mitochondrial protein was increased ~6-fold between the late foetal stage and the first day after birth. The scale of this increase is in accord with

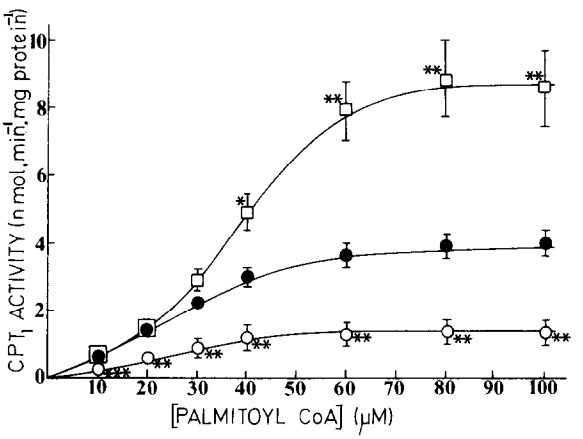

Fig. 1. Effect of palmitoyl CoA concentration on CPT_1 activities in foetal, neonatal and pregnant maternal rat liver mitochondria. L-Carnitine was present throughout at 400 μM. The values are means of 4 expt in each case. The bars indicate SEM: (○) foetal; (●) pregnant maternal; (□) neonatal; *, **, *** indicate $P < 0.02$, < 0.01 and < 0.001, respectively *vs* the maternal activity. Hill plots of the data from each of the individual experiments were in all cases good-fit straight lines (0.93< and <0.99) and were used to obtain n_H and $S_{0.5}$ values (the concentration of palmitoyl CoA giving half-maximal CPT_1 activity) by regression analysis. These values were: foetal, $n_H = 2.23 \pm 0.09$, $S_{0.5} = 23.4 \pm 1.0 \mu$M; pregnant maternal, $n_H = 2.28 \pm 0.08$, $S_{0.5} = 23.5 \pm 0.9 \mu$M; neonatal, $n_H = 2.68 \pm 0.05$ ($P < 0.01$ *vs* foetal), $S_{0.5} = 32.6 \pm 2.8 \mu$M ($P < 0.05$ *vs* foetal).

that reported in [12,15,16] in which similar assay methods were used. Since we have shown [37] that hepatic CPT_1 activity in fed pregnant rats is similar to that in non-pregnant adults, the maternal activity shown in fig. 1 may be taken as indicative of the normal adult activity. The CPT_1 activity in neonatal animals was about twice that of the adult value which, again, is in accord with earlier studies [15,16]. The n_H and the $S_{0.5}$ for palmitoyl CoA observed under the present assay conditions for the enzyme in foetal rats were essentially identical with those for the adult. However, both these parameters were significantly increased in the neonatal liver (legend to fig. 1). As a result of these changes, differences in the neonatal CPT_1 activity compared with those in the foetus or the adult are particularly accentuated at higher [palmitoyl CoA]. Previous studies have not investigated the relationship of

Table 1

CPT_2 activities (nmol.min^{-1}.mg protein^{-1}) in foetal, neonatal and pregnant maternal rat liver mitochondria

	[palmitoyl CoA]			
	20 μM	40 μM	60 μM	80 μM
Foetal	3.79 ± 0.57	6.39 ± 0.60	5.64 ± 0.14	3.91 ± 0.47
	(6.53)	(5.71)	4.59)	2.81)
Neonatal	3.19 ± 0.60	5.81 ± 0.42	4.61 ± 0.71	3.07 ± 0.59
	(2.16)	(1.17)	(0.58)	(0.39)
Pregnant	4.73 ± 0.48	7.50 ± 0.82	6.98 ± 0.70	4.95 ± 0.50
maternal	(3.28)	(2.48)	(1.90)	(1.25)

L-Carnitine was present throughout at 400 μM. Measurements were made with the same preparations of mitochondria used for fig. 1. The values are means ± SEM of 4 expt in each case. CPT_2 activity was calculated as in section 2. The values in parentheses represent the ratio CPT_2/CPT_1

activity to [palmitoyl CoA] in these different states.

Table 1 shows that these changes in CPT activity were confined to the overt, CPT_1 activity. The latent, CPT_2, activity did not differ significantly between the 3 states at any of the 4 tested [palmitoyl CoA]. This is essentially in accord with [38] but at variance with [16]. The observation that these CPT_2 activities exhibited quite different profiles of activity *vs* [palmitoyl CoA] from those of CPT_1 (fig. 1) is in accord with other studies [39]. In essence, a substantially smaller proportion of the total CPT activity is overt in the foetal liver compared with the adult, whereas the opposite is observed in the neonatal animal.

foetal to neonatal transition was accompanied by a substantial decrease in sensitivity to malonyl CoA (fig. 2). Under the present assay conditions ([palmitoyl CoA] and [albumin] both = 20 μM), 50% inhibition of CPT_1 was obtained with 0.6 μM in the foetal state whereas 9 μM malonyl CoA was necessary to achieve the same effect in the neonate. In fed adult animals, pregnancy may not greatly alter sensitivity to malonyl CoA [29,37]. Therefore, 5 μM malonyl CoA, which is necessary to achieve 50% inhibition of the pregnant maternal CPT_1 may also be taken as indicative of the situation in the non-pregnant adult. We conclude that a decrease (~14-fold) in sensitivity of CPT_1 to malonyl CoA is a further mechanism participating in the rapid development of fatty acid oxidative

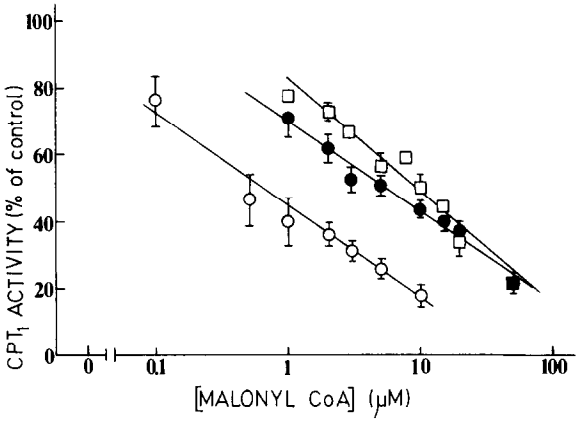

Fig. 2. Effect of malonyl CoA concentration on CPT_1 activities in foetal, neonatal and pregnant maternal rat liver mitochondria. L-Carnitine was present throughout at 400 μM and palmitoyl CoA at 20 μM. The values are means ± SEM for 4 expt except where no error bar is shown, in which case the values are the means of 2 expt. The values are expressed as percentages of the activities in the absence of malonyl CoA which may be deduced from fig. 1: (○) foetal; (●) pregnant maternal; (□) neonatal.

and ketogenic capabilities at birth. This change, coupled with the substantial increase in CPT_1 specific activity and the probable decrease in hepatic malonyl CoA content (see section 1) represents a powerful metabolic switching mechanism.

The hormonal, or other, factors responsible at birth for the increase in CPT_1 activity and the decrease in malonyl CoA sensitivity are unknown. It is of interest however that the newborn rat is relatively hypothyroid [40,41] since the maturation of the hypothalamic–pituitary axis mainly occurs during the neonatal period. The foetal rat is similar to the hypothyroid adult in having a low CPT_1 activity but a 'normal' CPT_2 activity [30]. Furthermore, in the hypothyroid adult, the increase in CPT_1 activity in response to fasting is exaggerated [30]. Whether the glucose to fatty acid nutritional change together with the decrease in insulin/glucagon ratio at birth provides this system with a stimulus similar to that of fasting remains to be established.

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